

COMPARATIVE KINETICS AND EFFECT OF DIFFERENT MEDIA ON THE GROWTH OF THREE FOLLICOLOUS FUNGI *ALTERNARIA ALTERNATA*, *TRICOTHECIUM ROSEUM* AND *FUSARIUM OXYSPORIUM*

RAFIQ AHMAD DAR, A. N. RAI & JAISHRI SURYWANSHI

Mycology and Plant Pathology Laboratory, Department of Botany, Dr Hari Singh Gour

Central University, Sagar, Madhya Pradesh, India

ABSTRACT

Three follicolous fungi *Alternaria alternata*, *Tricothecium roseum* and *Fusarium oxysporium* were collected on different hosts from different locations in South Kashmir (India) and their comparative growth kinetics and effect on growth was observed using different growth media- modified Potato Dextrose Agar (PDA with added Biotin), 6-Benzyl Amino Purine (6-BAP, Kinetin) and Malt Extract Agar (MEA). Responses to the media appeared to be related both to inherent properties of the fungi and to their natural ecological behavior. The present study is concerned to analyze the comparative growth kinetics and effect of external supplement of Biotin (Vitamin H), Malt extract and Kinetin (a plant hormone) on the growth rates of three different fungi. Biotin-binding proteins (BBPs) have been identified from different organisms including bacteria, birds, amphibians, and recently also in fungi. BBPs have been suggested as broad range antimicrobial agents by forming a biotin-free zone. Besides its antimicrobial and insecticidal activity and the recent evidence for a protein-mediated defense of fungi against predators and parasites it also shows profound effect on the fungal growth of certain species.

The effect of plant growth regulators have been intensively investigated especially in rusts. Many reports have noted a positive relationship between changes in fungus morphology and exogenously applied growth regulators. Different fungi respond differently to exogenously added vitamin (Biotin) and plant growth hormone (Kinetin) media. Maximum growth rate shown by different logistic and exponential curves corresponds to *Alternaria alternata* on Biotin- PDA medium and minimum rate corresponds to *Tricothecium roseum* on 6-BAP medium (plant hormone medium) while as the growth of *Fusarium oxysporium* remains almost unchanged in changing the media from Biotin-PDA to 6-BAP. It reflects that biotin (added vitamin) shows predominant effect on the growth of certain fungi like *Alternaria alternata*, *Fusarium oxysporium* on solid culture medium in contrast to plant hormone, kinetin which shows least effect.

KEYWORDS: Follicolous Fungi, Potato Dextrose Agar, 6- Benzyl Amino Purine, Malt Extract Agar, Biotin, BBPS, Kinetin, Ecological Behavior

INTRODUCTION

Microorganisms are capable of growing on a wide range of substrates and can produce a broad spectrum of products. The medium had a significant effect on the growth rates of the fungi as well as the production of, and response to, volatile and non-volatile antibiotic compounds and hyphal interactions. When a nutrient medium is inoculated with a microorganism, the growing culture passes through a number of the phases. Initially, growth does not occur and this period is referred to as the lag phase, while may be considered a period of adaptation. Following an interval during which the growth rate of the cells gradually increases, the cells divide at a constant, maximum rate and this period is referred to as the

log or exponential phase. Exponential growth cannot continue indefinitely, because the medium is soon depleted of nutrients and enriched with metabolites. Several enzymes are synthesized in the exponential phase, while many secondary metabolites emerge in the ensuing stationary phase, when there is no net growth.

Up to this time, attempts have been made to characterize the kinetics of growth in fungi but have been hampered by the inability to obtain homogeneous pure cultures. Specifically, aggregation of germinating spores, hyphal intertwining, and the presence of various developmental stages within a single mycelial mat contribute to the heterogeneity of culture samples (G. Dorn, 1965). In 1963, Mackintosh and Pritchard observed the formation of microcolonies when *Aspergillus nidulans* was grown on solid agar medium in the presence of surface-active compounds such as sodium dodecyl sulfate (SDS) or sodium deoxycholate (SDC). These agents, although restricting radial growth of this fungus, did not appear to affect its viability or normal differentiation. Present work is an attempt to analyze the effect on growth of three follicolous fungi, *Alternaria alternata*, *Tricothecium roseum* and *Fusarium oxysporium* using Potato Dextrose Agar with added vitamin H (modified PDA), 6-Benzyl Amino Purine (6-BAP, phyto hormone) and Malt Extract Agar (MEA). Biotin is a vitamin required by all prokaryotes and eukaryotes as an essential cofactor of several carboxylases involved in central metabolic pathways. Avidin, for instance, has been proposed as antimicrobial host-defense factor against pathogen infections in chicken, as it inhibits the growth of some microorganisms, and is induced in different tissues in chicken upon injury and bacterial and viral infection. Kinetin (6-furfurylaminopurine) is a highly potent growth factor that, along with other plant growth substances, promotes cell division and ensures orderly growth and development of plants. Kinetin has been shown capable of delaying or preventing a host of age-related changes of human skin fibroblasts grown in laboratory culture.

The growth rate is shown by different logistic and exponential curves reflecting different developmental stages of fungus.

$$\text{Rate} = \frac{\Delta \text{in some measure of growth}}{\Delta t}$$

Comparisons of reproductive growth and non reproductive growth involve different practical measures:

- Linear dimensions
- Mass or weight
- Volume
- Metabolic activity
- Cell constituent (e.g. protein)
- Absorbance or transmittance

Trinci (1969) had studied the kinetics of hyphal extension and branch initiation of different fungal mycelia and found the result support the hypothesis that mycelia growth involves the duplication of "growth unit" containing of a tip and certain mean length of hyphae. Trinci has defined the hyphal growth unit as the mean length of hypha which contributes protoplasm to the extension of the tips of a mycelium, while the peripheral growth zone is the maximum length of hypha contributing to the extension of the leading hyphal tips of a mature colony. The radius of fungal colonies and the length of unbranched hyphae (Trinci & Banbury, 1967) increase linearly with time (i.e. they grow at a constant rate, Kr). Thus

$$R_1 = R_0 + Kr (t_1 - t_0) \quad (1)$$

Where R_1 = colony radius or hyphal length at time t_1 , R_0 = colony radius or hyphal length

At t_0 , and Kr = the growth rate constant. However, filamentous fungi grow exponentially in submerged shake-flask culture (Zalokar, 1959; Trinci, 1969) as long as all nutrients are present in excess and growth inhibitors are not accumulated. Thus

$$\frac{dM}{dt} = \alpha M \quad (2)$$

Where M = organism mass per unit volume, t = time, and α is a constant known as the specific growth rate. Integration of equation (2) gives

$$\ln M_1 = \ln M_0 + \alpha (t_1 - t_0) \quad (3)$$

Where \ln = natural logarithm, M_0 = mould dry weight at time t_0 , and M_1 = mould dry weight at time t_1 . Thus through hyphal branching the observed linear increase in colony Radius (or length of unbranched hypha) is compatible with the exponential nature of the Organism's growth in submerged culture. Specific growth rate is a direct measure of the

Exponential rate of mould growth and is related to the organism's doubling time (T_d) by the expression,

$$T_d = \frac{\ln 2}{\alpha}$$

MATERIALS AND METHODS

Collection of Specimens: The three follicolous fungi *Alternaria alternata*, *Tricothecium roseum* and *Fusarium oxysporium* were collected (on 15 september, 2013) in the South Kashmir from three different locations namely Dialgam, Achabal and Pahalgam respectively from the leaves of Apple trees and Onion shoot tips using sterile poly bags. The collected specimens were pressed and preserved using naphthalene to avoid microbial decomposition.

Laboratory Operation: The collected specimens have been identified and studied using all microscopic aids, chemical reagents (lactophenol, 3% KOH, 1% cotton blue) and relevant literature.

Production of Heterogeneous Poly Cultures: The isolated fungi were inoculated using sterile loop on three different growth media (PDA with added vitamin H, 6-BAP and MEA) and bottom of each Petri plate was labeled. The buffer (HIMEDIA) and streptomycin (Ranbaxy) tablets were added to media to control pH(4-5) and bacterial contamination respectively. The 09 labeled culture plates were incubated (REMI) at 28°C for 10 days.

Production of Homogenous Monocultures: Sub culturing of heterogeneous poly cultures were carried out following above mentioned protocol with high aseptic conditions. The 09 labeled pure cultures were incubated again at 28°C for 60 days and subsequent radial colony growth was noted down regularly after 5 day intervals.

RESULTS AND DISCUSSIONS

A **log phase** was observed in all cultures within the first 24 h of incubation. The logarithmic growth phase started on day 2. Maximum growth was reached on day 15 in *Alternaria alternata* and *Fusarium oxysporium*, but *Tricothecium roseum* showed maximum growth on day 21, followed by the decline phase. The growth curves of the *Alternaria alternata* and *Fusarium oxysporium* generally followed a homogenous pattern comparable to the growth curve of *Tricothecium roseum*. No **stationary phase** could be established with precision for any of the culture analyzed nor for the mean growth

curve. The **lag phase** shows little or no cell division. However, metabolic activity is intense.

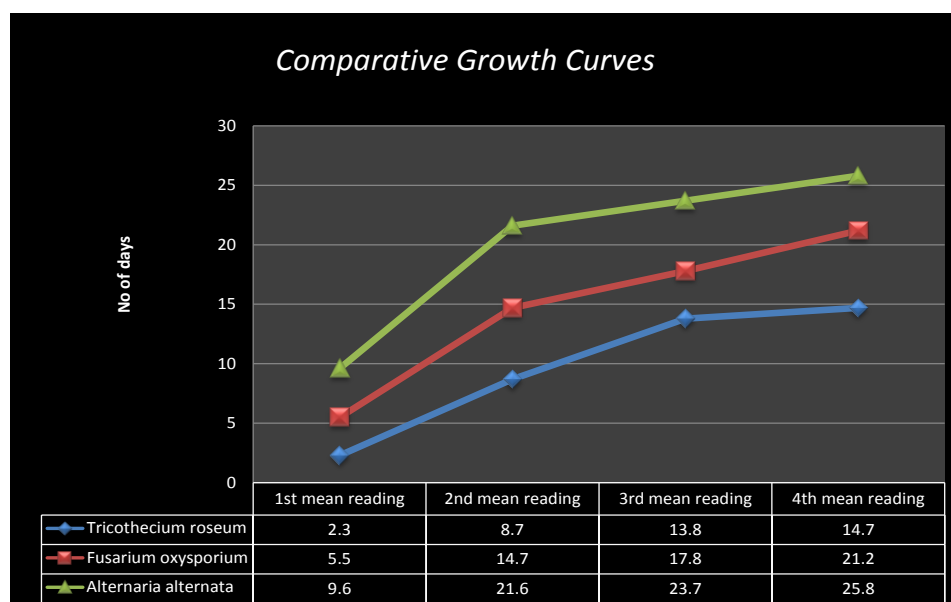


Figure 1: Growth Curves of Three Follicolous Fungi (Mean of Four Determinations), Based on Mycelial Radial Diameter, Utilizing Biotin-PDA, MEA & 6-BAP Growth Media, Incubated at 28°C, for 60 Days

In the **log phase**, the cells are reproducing most actively, and their generation time reaches a minimum and remains constant; a logarithmic plot produces an ascending straight line. They are then most active metabolically and most sensitive to adverse conditions. In a chemostat, it is possible to keep a population in such exponential growth indefinitely. Without a chemostat, however, microbial deaths eventually balance numbers of new cells, and a **stationary phase** is reached.. When the number of deaths exceeds numbers of new cells formed, the **death phase**, or **logarithmic decline**, is reached. Analysis of Figure 1. showed the presence of a lag phase in the 09 cultures studied up to 24 h of culture. This phase is usually observed when fresh medium is inoculated with cells derived from an old culture. Even if all microorganisms are viable, the cells might be deprived of enzymes so that the total growth rate can only be reached when the optimum concentrations of these substances for synthesis are restored. With respect to the logarithmic growth phase, the 09 cultures showed maximum growth between day 33 and day 37, a period when all conidia were found to be viable.

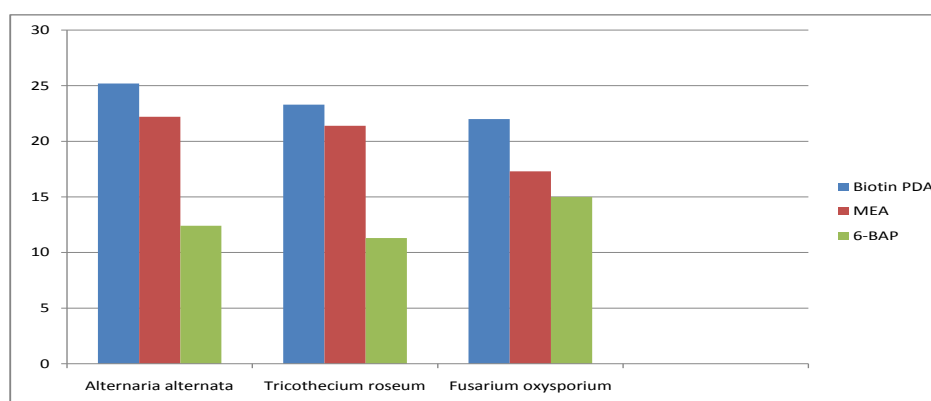


Figure 2: Showing the Growth Rate Variations in Different Media. Maximum Growth Rate Corresponds to *Alternaria alternata* on Biotin- PDA and Minimum Rate Corresponds to *Tricothecium roseum* on 6-BAP

Interestingly, according to the literature, production of mycotoxins by the fungus probably starts during this

period (Stevenson Rb, 1974). Also during the logarithmic growth phase, the percentage of viable conidia reached its maximum level on day 40. Regarding the decline phase which started on day 44, some investigators emphasize that the conditions that control this phase are the same that interfere with the duration of the logarithmic growth phase. The sporadic increases in the number of viable cells observed during the decline phase might be attributed to autolysis, which results in the release of nutrients that can be utilized by viable cells.

Table 1: Mean Radial Growth Rates (mmd⁻¹) per 5 Days of Three Follicolous Fungi on Three Different Media, Biotin -Potato Dextrose Agar (PDA), Malt Extract Agar (MEA) & 6-Benzyl Amino Purine (6-BAP) Incubated at 28°C

Sn	<i>Alternaria alternata</i>			<i>Fusarium oxysporium</i>			<i>Tricothecium roseum</i>		
	PDA	MEA	6-BAP	PDA	MEA	6-BAP	PDA	MEA	6-BAP
1	4.5	3.2	1.5	2.5	1.5	1.0	1.5	1.2	0.8
2	9.6	5.4	3.7	5.5	2.3	1.8	2.3	1.9	1.4
3	12.3	7.7	5.3	7.4	4.6	2.3	4.7	2.3	2.0
4	16.4	11.6	8.1	11.3	7.7	5.5	7.3	4.7	3.5
5	21.6	16.6	12.4	14.7	11.6	7.1	8.7	6.3	4.1
6	23.7	20.4	15.7	17.8	13.6	9.2	13.8	8.3	6.5
7	24.2	21.9	17.6	19.7	16.6	11.4	14.0	11.4	8.3
8	25.8	23.6	19.3	21.2	18.2	13.5	14.7	12.6	9.7

Analysis of figure 1, 2, 3 shown below reflects that media had significant effect on the growth rates of these three follicolous fungi. *Alternaria alternata* grows vigorously in biotin-PDA media but its growth is relatively hampered in 6-BAP media while as the growth rate of *Fusarium oxysporium* remains almost unchanged in changing the media from biotin-PDA to 6-BAP. Similarly *Tricothecium roseum* shows ideal growth rate in biotin-PDA but shows almost negligible growth in 6-BAP.

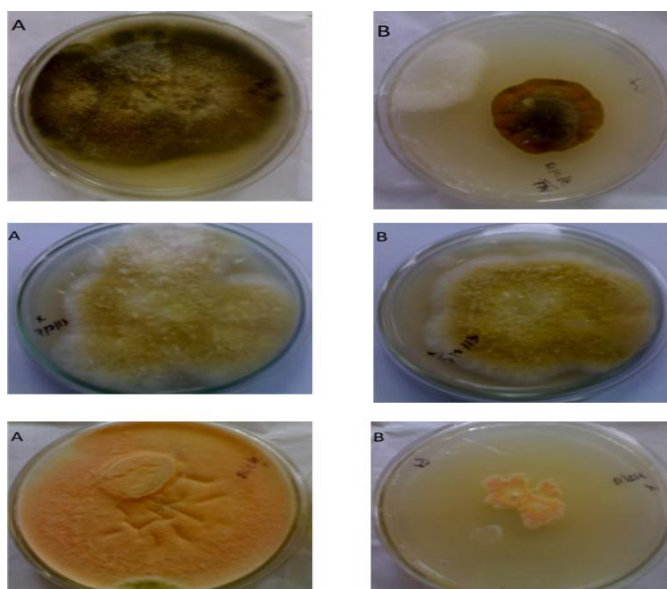


Figure 3,4,5: Shows 10 Days Old Cultures (A) & (B) of *Alternaria alternata*, *Fusarium oxysporium* & *Tricothecium roseum* Incubated at 28 C on Biotin-PDA & 6-BAP Growth Media Respectively Reflects that Three Follicolous Fungi Respond Differently to Different Growth Media

CONCLUSIONS

- Media had significant effect on the growth rates of these three follicolous fungi. *Alternaria alternata* grows vigorously in biotin-PDA media but its growth is relatively hampered in 6-BAP media while as the growth rate of *Fusarium oxysporium* remains almost unchanged in changing the media from biotin-PDA to 6-BAP. Similarly *Tricothecium roseum* shows ideal growth rate in biotin-PDA but shows almost negligible growth in 6-BAP.
- The growth curves of the *Alternaria alternata* and *Fusarium oxysporium* generally followed a homogenous pattern comparable to the growth curve of *Tricothecium roseum*.
- Protein synthesized behind tip contributes to elongation rate (translocation).
- Need a minimum of 12 mm of hyphal length to sustain an elongation rate of 100µm/min
 $100\ \mu\text{m} = 12\ \text{mm length}$
 $100\ \mu\text{m (per min extension rate)} \times 120\ \text{min (time to double protein)} = 1200\ \mu\text{m} = 12\ \text{mm}$
- Phytohormone, kinetin showed predominant effect on the growth of *Fusarium oxysporium* only in contrast to biotin.
- Exogenous supplement of vitamins accelerates the growth rates of certain fungi like *Alternaria alternata*, *Fusarium oxysporium* in comparison to phytohormones whose effect is seen less on the growth of *Tricothecium roseum*.

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